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A METHOD FOR SAMPLING YEASTS AND BACTERIA IN SOUND WOOD

by

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ABSTRACT

A tubular chisel is described and methods are presented for sampling populations of yeasts and bacteria within the sound wood of living trees.

Good data on the numbers and kinds of unicellular organisms, including yeasts and bacteria, within undecayed wood are needed to evaluate their role in causing diseases of wood, in microbial successions, and in formation of fermentation byproducts in logs, considered important as insect attractants.[1/]

The method described here, originally developed for sampling aspen logs,[2/] has proven useful for quantitative sampling of unicellular organisms in undecayed wood. Cores of wood are extracted with a hollow, cylindrical chisel. The sap from the wood cores is aseptically expressed, diluted, and plated on agar media.

1/ K. Graham. Anaerobic induction of primary chemical attractancy for Ambrosia beetles. Can. J. Bot. 46: 905-908. 1968.

2/ Donald Maurice Knutson. Wetwood in trembling aspen (*Populus tremuloides* Michx.). 152 pp. 1968. (Ph.D. thesis, Univ. Minn., School of Forestry.

THE METHOD

The chisel consists of a hollow steel cylinder sharpened on one end and with a flattened lip on the other (fig. 1). It is 8 centimeters long and has a 1.3-centimeter (1/2-inch) inside diameter at the cutting edge.

With the chisel, wood cylinders are taken from disks of wood (with the grain) (fig. 2). The sampling method and analysis involve these steps:

1. Bolts, approximately 1 meter long, are cut from chosen trees, sealed in plastic bags, taken immediately to the laboratory, and frozen until needed. Drying must be minimized since the method depends on extraction of sap.

2. Disks of wood, approximately 5 to 6 centimeters thick, are cut from the center of the bolt with a chain saw. To reduce contamination of the cut wood surface, the bark should be removed before cutting the disk.

3. Sampling points can be chosen and replicated in a disk in any variety of designs, depending on sampling objectives. For example, one can compare the number of organisms from different zones of sapwood or contrast sapwood with heartwood organisms.

4. Cylinders are cut by driving the chisel along the grain of the selected sampling point while the wood disks are frozen, to facilitate cutting and prevent changes in moisture content of the wood. The chisel is dipped in 95 percent alcohol and flamed for each operation. The cylinders are easily removed from the chisel because of the larger diameter inside the chisel above the cutting edge (fig. 1).

5. Both ends of each cylinder are cut off with a hacksaw or bandsaw blade which has been dipped in alcohol and flamed. The cylinders should be reduced to an established length (3-4 centimeters). After the individual cylinder is cut, it is held with a forceps, flamed with 95 percent alcohol, and placed in a presterilized length (15 centimeters) of rubber tubing (1.3-centimeter (1/2-inch) inside diameter).

6. The rubber tubing containing the wood cylinder is placed between the platens of a Carter Laboratory Press[3/] and pressed at 3,000 pounds per square inch (p.s.i.). The press is positioned at a slant so that the sap will run down the tubing into a sterile vial.

[3/] The use of proprietary or brand names does not imply approval of the product to the exclusion of others which may also be suitable.

7. The expressed sap is diluted by standard dilution techniques and cultured on appropriate medium. Difco Nutrient Agar is a good standard medium. Petri dishes are incubated at 25-30° C. for 3 days before colonies are counted. Since many colonies will be small, counts should be made under magnification. Data can be expressed as the number of colonies per milliliter of expressed sap, or related to volume of wood sampled.

Although the data cannot be considered the total number of organisms, this method does provide a means of quantifying relative numbers of microbes in an environment which is very difficult to study--the inside of a tree.

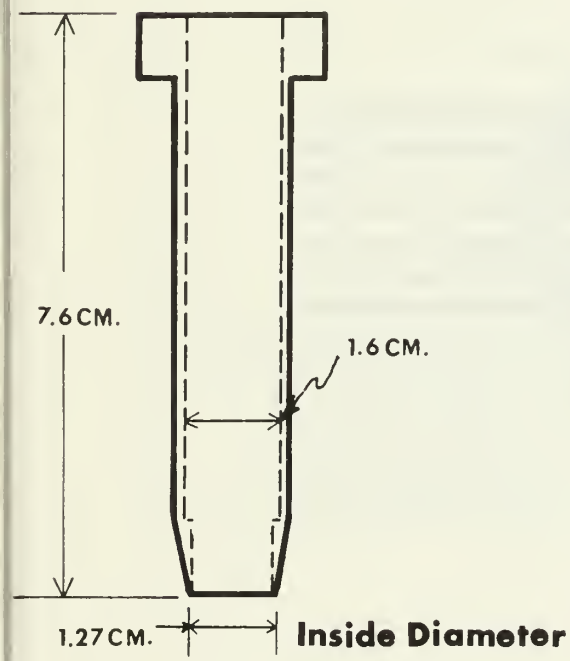

Figure 1.--*Hollow circular steel chisel for taking wood cylinders.*

Figure 2.--*Method of taking wood cylinders with the chisel.*

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